Hydrothermal Deamidation of 4-N-Acylcytosine Nucleoside Derivatives: Efficient Synthesis of Uracil Nucleoside Esters

Ireneusz Nowak and Morris J. Robins*

Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah 84602-5700

morris_robins@byu.edu

Supporting Information

Pages S2–S5: Experimental procedures and characterization data.

Pages S6–S12: $^{13}$C NMR spectra of 1f, 1j, 2a–e, 2g–i, 3g–j (2c, 2f, or 2j is the same derived compound; 3f or 3j is the same derived compound).
Experimental

General: $^1$H (500 MHz) and $^{13}$C (125 MHz) NMR spectra were recorded in CDCl$_3$ unless otherwise noted. $^{13}$C NMR peaks with identical chemical shifts for more than one carbon are specified, and overlapping peaks for multiple carbons are indicated by a shift range (ovlp). Reagent grade chemicals were used. Deionized water was used for all deamination experiments.

4-N-Pivalyl-2',3',5'-tri-O-benzoylcytidine (1f). A solution of 2',3',5'-tri-O-benzoylcytidine$^1$ (700 mg, 1.26 mmol), pivalic anhydride (1.5 mL), and pyridine (2.0 mL) was stirred at 110 °C for 19 h. Volatiles were evaporated, and the residue was chromatographed (EtOAc/hexanes, 1:3 → 2:1) to give crystalline material. Recrystallization (CH$_2$Cl$_2$/MeOH) provided 730 mg (93%) of 1f: mp 130–132 °C; $^1$H NMR δ 1.27 (s, 9H), 4.71–4.87 (m, 3H), 5.78 (t, $J$ = 5.4 Hz, 1H), 5.90 (t, $J$ = 5.4 Hz, 1H), 6.49 (d, $J$ = 4.9 Hz, 1H), 7.36–7.64 (m, 10H), 7.89–8.11 (m, 8H); $^{13}$C NMR δ 26.8, 40.1, 63.5, 70.8, 74.5, 80.4, 89.1, 96.8, 128.2–129.7 (ovlp), 133.4, 144.1, 154.8, 162.5, 164.9, 165.0, 165.9, 177.8.

4-N-Trifluoroacetyl-2',3',5'-tri-O-benzoylcytidine (1j). Trifluoroacetic anhydride (0.19 mL, 0.28 g, 1.35 mmol) was added dropwise to a cold (0 °C), stirred solution of 2',3',5'-tri-O-benzoylcytidine$^1$ (500 mg, 0.90 mmol) and pyridine (0.5 mL) in CH$_2$Cl$_2$ (5 mL), and stirring was continued for 15 min. Volatiles were evaporated, and the residue was chromatographed (EtOAc/hexanes, 1:2) to give 1j (500 mg, 85%): mp 187–190 °C (CH$_2$Cl$_2$/MeOH); $^1$H NMR δ 4.69–4.89 (m, 3H), 5.84 (t, $J$ = 5.4 Hz, 1H), 5.90 (t, $J$ = 5.4 Hz, 1H), 6.36 (d, $J$ = 4.4 Hz, 1H), 6.53 (d, $J$ = 6.8 Hz, 1H), 7.35–7.64 (m, 9H), 7.91–8.09 (m, 7H), 10.97 (br s, 1H); $^{19}$F NMR δ –77.1 (s, 3F); $^{13}$C NMR (DMSO-$d_6$) δ 63.5, 70.3, 73.9, 79.3, 91.0, 98.2, 116.0 (q, $J$ = 288.8 Hz), 128.5–129.5 (ovlp), 113.6, 133.9, 134.0, 147.0, 155.0, 164.3, 164.56, 164.62, 165.5.

General Procedure for Acylation and Solvolysis of the Resulting N,O-Peracyl Cytosine Derivatives. Method A (arylcarboxylic acid chlorides as acylating agents). Toluyl chloride (10.0 mL, 10.2 g, 65.8 mmol) was added to a stirred suspension of 2'-deoxycytidine hydrochloride (6.0 g, 21.3 mmol) in dried pyridine (40 mL), and stirring was continued at ambient temperature for 3 h. The semisolid residue was partitioned between H$_2$O (50 mL) and

---

CH$_2$Cl$_2$ (100 mL). The aqueous phase was extracted (CH$_2$Cl$_2$, 50 mL), and the combined organic phase was dried (MgSO$_4$). CH$_2$Cl$_2$ was evaporated at ambient pressure, MeOH (3 mL) was added, and the solution was heated at 80 °C (oil-bath temperature) for 1 h. Volatiles were evaporated, and the residue was transferred to a 500-mL flask equipped with Teflon valve. DME (75 mL) and H$_2$O (50 mL) were added, and the flask was placed in a preheated oil bath at 125 °C. Stirring was continued for 12 h at 125 °C, and the flask was removed from the oil bath and allowed to cool to ambient temperature (crystallization occurred throughout the entire solvent volume). The flask was then placed in a refrigerator (4 °C) for 1 h. The product was filtered, washed with cold H$_2$O/MeOH (1:1, 30 mL), and dried under vacuum to give 2'-deoxy-3',5'-di-O-(4-methylbenzoyl)uridine$^2$ (2b) (8.24 g, 84%).

Method B (aliphatic acid anhydrides as acylating agents). A suspension of cytidine (300 mg, 1.23 mmol), propionic anhydride (0.8 mL, 0.81 g, 6.2 mmol), and pyridine (2 mL) was stirred for 2 h at 80 °C. Volatiles were evaporated, and the viscous oil was dried at 90 °C under vacuum. A solution of this material in DME (4.5 mL) and H$_2$O (3 mL) was heated in a sealed flask for 9 h at 125 °C. Volatiles were evaporated, and chromatography (EtOAc/hexanes, 1:2 → 3:1) of the residue gave 2',3',5'-tri-O-propionyluridine (2h) (49%) as a colorless oil. Further elution [EtOAc → EtOAc/MeOH (10:1)] gave 2',3',5'-tri-O-propionylcytidine (3h) (30%) as a colorless oil.

3',5'-Di-O-benzoyl-2'-deoxyuridine$^3$ (2a). mp 225–226 °C (MeOH); $^1$H NMR $\delta$
2.30–2.36 (m, 1H), 2.77 (ddd, $J = 1.9, 5.8, 14.2$ Hz, 1H), 4.55–4.57 (m, 1H), 4.71 & 4.74 (dd, $J = 3.9, 12.2$ Hz, 2 × 1H), 5.60 (ddd, $J = 1.9, 7.8$ Hz, 1H), 5.63 (dt, $J = 6.9, 1.9$ Hz, 1H), 6.42 (dd, $J = 5.9, 8.3$ Hz, 1H), 7.46–8.08 (m, 11H), 8.69 (br s, 1H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 36.1, 64.4, 74.7, 81.2, 84.9, 102.2, 128.8–129.5 (ovlp), 133.6, 133.7, 140.6, 150.4, 163.1, 165.3, 165.6.

2'-Deoxy-3',5'-di-O-(4-methylbenzoyl)uridine$^2$ (2b). mp 214–216 °C (CH$_2$Cl$_2$/MeOH); $^1$H NMR $\delta$
2.27–2.33 (m, 1H), 2.43 (s, 3H), 2.44 (s, 3H), 2.75 (ddd, $J = 1.5, 5.4, 14.2$ Hz, 1H), 4.53–4.56 (m, 1H), 4.68 & 4.73 (dd, $J = 3.4, 12.2$ Hz, 2 × 1H), 5.58–5.62 (m, 2H), 6.41 (dd, $J = 5.8, 8.3$ Hz, 1H), 7.25–7.29 (m, 4H), 7.53 (d, $J = 8.3$ Hz, 1H), 7.89 & 7.95 (2 × d, $J = 8.3$ Hz, 2 ×

2H), 8.87 (br s, 1H); \(^{13}\)C NMR (DMSO-\(d_6\)) \(\delta\) 21.20, 21.22, 64.2, 74.5, 81.3, 84.9, 102.2, 126.5, 126.6, 129.3–129.5 (ovlp), 140.5, 143.9, 144.1, 150.4, 163.0, 165.3, 165.5.

**2',3',5'-Tri-O-benzoyluridine**\(^4\) (2c). mp 142–144 °C (toluene); \(^1\)H NMR \(\delta\) 4.66–4.86 (m, 3H), 5.62 (dd, \(J = 2.0, 8.3\) Hz, 1H), 5.76 (t, \(J = 5.6\) Hz, 1H), 5.90 (dd, \(J = 4.4, 5.9\) Hz, 1H), 6.33 (d, \(J = 5.4\) Hz, 1H), 7.35–7.64 (m, 10H), 7.93–8.12 (m, 6H), 9.02 (br s, 1H); \(^{13}\)C NMR \(\delta\) 63.7, 71.0, 73.7, 80.3, 88.1, 103.3, 128.2–129.8 (ovlp), 133.5, 133.6, 133.7, 150.2, 163.2, 165.17, 165.21, 165.9.

**2',3',5'-Tri-O-(4-methylbenzoyl)uridine**\(^5\) (2d). mp 198–201 °C (CH\(_2\)Cl\(_2\)/MeOH); \(^1\)H NMR \(\delta\) 2.36, 2.39, 2.41 (3 × s, 3 × 3H), 4.62–4.84 (m, 3H), 5.60 (d, \(J = 7.8\) Hz, 1H), 5.73 (t, \(J = 5.9\) Hz, 1H), 5.87 (t, \(J = 5.1\) Hz, 1H), 6.37 (d, \(J = 5.9\) Hz, 1H), 7.15–7.28 (m, 6H), 7.43 (d, \(J = 8.3\) Hz, 1H), 7.83–8.00 (m, 6H), 9.70 (br s, 1H); \(^{13}\)C NMR \(\delta\) 21.6, 63.6, 71.0, 73.4, 87.5, 103.3, 125.5, 125.8, 126.3, 129.1–129.9 (ovlp), 139.5, 144.4, 144.47, 144.53, 150.2, 163.1, 165.2, 165.3, 166.0.

**2',3',5'-Tri-O-(4-chlorobenzoyl)uridine**\(^6\) (2e). mp 237–239 °C (CH\(_2\)Cl\(_2\)/MeOH); \(^1\)H NMR \(\delta\) 4.64–4.82 (m, 3H), 5.69 (dd, \(J = 1.4, 7.8\) Hz, 1H), 5.73 (t, \(J = 5.6\) Hz, 1H), 5.86 (t, \(J = 5.4\) Hz, 1H), 6.19 (d, \(J = 5.4\) Hz, 1H), 7.34–7.47 (m, 7H), 7.85–8.04 (m, 6H), 8.31 (br s, 1H); \(^{13}\)C NMR (DMSO-\(d_6\)) \(\delta\) 63.6, 70.5, 73.5, 78.5, 90.1, 102.3, 127.3, 127.4, 128.0, 128.9–129.0 (ovlp), 131.1–131.2 (ovlp), 138.6, 138.9, 139.0, 142.7, 150.4, 163.3, 163.9, 164.7.

**2',3',5'-Tri-O-isobutyryluridine** (2g). Colorless oil: \(^1\)H NMR \(\delta\) 1.56–1.24 (m, 18H), 2.55–2.66 (m, 3H), 4.30–4.42 (m, 3H), 5.31–5.35 (m, 2H), 5.79 (d, \(J = 8.3\) Hz, 1H), 6.10 (d, \(J = 5.4\) Hz, 1H), 7.44 (d, \(J = 8.3\) Hz, 1H), 9.09 (br s, 1H); \(^{13}\)C NMR \(\delta\) 18.32, 18.38, 18.42, 18.51, 18.58, 18.63, 33.3, 33.4, 33.6, 62.9, 69.8, 72.4, 80.0, 86.7, 103.0, 139.0, 150.2, 163.1, 175.32, 175.35, 176.0.

**2',3',5'-Tri-O-propionyluridine** (2h). Colorless oil: \(^1\)H NMR \(\delta\) 1.11–1.20 (m, 9H), 2.32–2.48 (m, 6H), 4.34–4.41 (m, 3H), 5.35–5.35 (m, 2H), 5.82 (d, \(J = 7.8\) Hz, 1H), 6.10 (d, \(J = 4.9\) Hz, 1H), 7.48 (dd, \(J = 1.0, 8.3\) Hz, 1H), 19.15 (br s, 1H); \(^{13}\)C NMR \(\delta\) 8.6, 8.7 (2C), 26.8, 27.0, 27.2, 63.0, 69.9, 72.4, 86.9, 103.1, 139.2, 150.3, 163.2, 172.9 (2C), 173.5.

---


2',3',5'-Tri-\textit{O}-acetyluridine\textsuperscript{7} (2i). Colorless oil: \textit{\textsuperscript{1}H NMR} $\delta$ 2.11, 2.14, 2.15 (3 × s, 3 × 3H), 4.33–4.39 (m, 3H), 5.32–5.36 (m, 2H), 5.81 (d, $J = 7.8$ Hz, 1H), 6.06 (d, $J = 4.9$ Hz, 1H), 7.41 (d, $J = 8.3$ Hz, 1H), 9.51 (s, 1H); \textit{\textsuperscript{13}C NMR} $\delta$ 20.4, 20.5, 20.8, 63.1, 70.1, 72.7, 79.9, 87.4, 103.4, 139.3, 150.2, 162.9, 169.6 (2C), 170.1.

2',3',5'-Tri-\textit{O}-isobutyrylcytidine (3g). Colorless oil: \textit{\textsuperscript{1}H NMR} $\delta$ 1.15–1.22 (m, 18H), 2.53–2.67 (m, 3H), 4.30–4.36 (m, 3H), 5.40 (t, $J = 5.6$ Hz, 1H), 5.45 (dd, $J = 4.4, 5.4$ Hz, 1H), 5.94 (d, $J = 4.4$ Hz, 1H), 6.01 (d, $J = 7.3$ Hz, 1H), 6.81 (br s, 1H), 7.40 (d, $J = 7.8$ Hz, 1H), 8.47 (br s, 1H); \textit{\textsuperscript{13}C NMR} $\delta$ 18.4–19.2 (6C), 33.6, 33.7, 33.9, 63.1, 69.9, 73.2, 76.7, 89.6, 96.1, 140.7, 155.6, 166.1, 175.6, 175.8, 176.5.

2',3',5'-Tri-\textit{O}-propionylycytidine (3h). Colorless oil: \textit{\textsuperscript{1}H NMR} $\delta$ 1.11–1.18 (m, 9H), 2.30–2.46 (m, 6H), 4.30–4.40 (m, 3H), 5.43 (t, $J = 5.6$ Hz, 1H), 5.48 (dd, $J = 4.4, 5.8$ Hz, 1H), 5.93 (d, $J = 4.4$ Hz, 1H), 6.02 (d, $J = 7.3$ Hz, 1H), 6.84 (br s, 1H), 7.39 (d, $J = 7.8$ Hz, 1H), 8.45 (br s, 1H); \textit{\textsuperscript{13}C NMR} $\delta$ 8.4, 7.66 (2C), 8.74, 26.90, 26.92, 27.2, 30.2, 62.4, 69.3, 73.5, 79.7, 88.8, 97.2, 143.7, 154.6, 163.2, 172.6, 172.7, 173.4, 174.9.

2',3',5'-Tri-\textit{O}-acetylcytidine\textsuperscript{7} (3i). Colorless oil: \textit{\textsuperscript{1}H NMR} $\delta$ 2.09, 2.10, 2.13 (3 × s, 3 × 3H), 4.29–4.41 (m, 3H), 5.40 (t, $J = 5.6$ Hz, 1H), 5.46 (dd, $J = 4.9, 5.9$ Hz, 1H), 5.92 (d, $J = 4.4$ Hz, 1H), 5.96 (d, $J = 7.8$ Hz, 1H), 6.49 (br s, 1H), 7.40 (d, $J = 7.3$ Hz, 1H), 8.14 (br s, 1H); \textit{\textsuperscript{13}C NMR} $\delta$ 20.38, 20.40, 20.7, 62.9, 69.8, 73.3, 78.9, 90.1, 96.2, 140.9, 155.6, 166.2, 169.5, 169.6, 170.4.

2',3',5'-Tri-\textit{O}-benzoylcytidine\textsuperscript{8} (3j). Colorless oil: \textit{\textsuperscript{1}H NMR} $\delta$ 4.63–4.81 (m, 3H), 5.85–5.88 (m, 2H), 5.97 (t, $J = 6.1$ Hz, 1H), 6.18 (d, $J = 7.3$ Hz, 1H), 6.51 (br s, 1H), 7.32–7.56 (m, 10 H), 7.90–8.10 (m, 6H), 8.18 (br s, 1H); \textit{\textsuperscript{13}C NMR} $\delta$ 63.6, 70.9, 74.3, 79.3, 91.1, 96.4, 128.2–129.7 (ovpl), 133.2, 133.4, 141.3, 155.6, 165.1, 165.3, 166.1, 1662.
